Functional Characterization of IL-17F as a Selective Neutrophil Attractant in Psoriasis

Hideaki Watanabe[1], Mio Kawaguchi[2], Sawa Fujishima[1], Miyoko Ogura[1], Satoshi Matsukura[3], Hiroko Takeuchi[3], Motoi Ohba[4], Hirohiko Sueki[5], Fumio Kokubu[6], Nobuyuki Hizawa[2], Mitsuru Adachi[3], Shau-Ku Huang[7] and Masafumi Iijima[1]

IL-17F is known to be involved in many inflammatory diseases, but its role in skin diseases has not been fully examined. Because IL-8 is involved in many skin diseases such as psoriasis, we investigated the production of IL-8 in normal human epidermal keratinocytes (NHEKs) stimulated by IL-17F, tumor necrosis factor-α (TNF-α), IL-17A, and control using real-time PCR and ELISA. The results showed that IL-17F induced production of IL-8 in NHEKs in a time-dependent manner. Interestingly, the amounts of IL-8 stimulated by IL-17F were much higher than those stimulated by TNF-α or IL-17A. Next, we confirmed that selective mitogen-activated protein kinase kinase inhibitors significantly inhibited IL-17F-induced IL-8 production. Moreover, mouse skin intradermally injected with IL-17F expressed high level of *IL-8* mRNA and induced ERK1/2 phosphorylation. Histological examination of mouse skin that was injected with IL-17F revealed marked neutrophilia in dermis and the infiltration was significantly inhibited by anti-IL-8 antibody. Finally, IL-17F expression in skin biopsy samples from psoriasis patients were examined by western blotting and ELISA. IL-17F was upregulated in lesional psoriatic skin compared with nonlesional skin. These results indicate that IL-17F may be involved in psoriasis via, in part, the activation of ERK1/2 and the induction of IL-8 in keratinocytes.

Journal of Investigative Dermatology (2009) **129,** 650–656; doi:10.1038/jid.2008.294; published online 2 October 2008

INTRODUCTION

Psoriasis is a chronic inflammatory skin disorder and is now recognized to be mediated in part by immune alterations. Psoriatic plaques are histologically recognized by typical patterns of abnormal epidermal hyperplasia and differentiation (Gottlieb *et al.*, 2005). In the past 20 years, it has become clear that these epidermal changes are secondary to robust immune activation within psoriatic plaques mediated by cytokine and chemokine release by activated inflammatory cells including T cells and neutrophils (Nickoloff *et al.*, 2007; van Beelen *et al.*, 2007). On the basis of the biochemical analyses and *in vitro* studies, it has become evident that IL-8 greatly contributes to the major pathologic changes seen in psoriasis (Sticherling *et al.*, 1991; Takematsu and Tagami, 1993; Duan *et al.*, 2001). However, its biological mechanisms remain incompletely defined.

IL-17F is involved in tissue inflammation by inducing the release of proinflammatory and neutrophil-mobilizing cytokines (Hymowitz *et al.*, 2001; Kawaguchi *et al.*, 2001; Starnes *et al.*, 2001). Furthermore, it has been documented that Th17 cells, which produce IL-17A and IL-17F, develop via signaling pathways that are independent of those required for Th1 or Th2 cells (Steinman, 2007). Although an increase of IL-17A has been reported in acute atopic dermatitis (Toda *et al.*, 2003), allergic contact dermatitis (Albanesi *et al.*, 1999) and psoriasis (Teunissen *et al.*, 1998; Albanesi *et al.*, 2000; Zheng *et al.*, 2007), the role of IL-17F in skin diseases has not yet been fully examined. Therefore, we investigated the functional role of IL-17F in normal human epidermal keratinocytes (NHEKs) in this study. Our results showed that IL-17F may be important in psoriasis.

RESULTS

IL-8 gene expression in NHEKs induced by IL-17F

To examine the expression of the *IL-8* gene in NHEKs, we investigated whether IL-17F increased the expression of mRNA for *IL-8* using quantitative reverse transcription real-time PCR. The expression of *IL-8* mRNA at 24 hours was 30- and 37-fold higher than that in the tumor necrosis factor-α (TNF-α) and IL-17A treated group, respectively (Figure 1a).

[1]*Department of Dermatology, Showa University School of Medicine, Tokyo, Japan;* [2]*Department of Respiratory Medicine, Institute of Clinical Medicine, University of Tsukuba, Ibaraki, Japan;* [3]*First Department of Internal Medicine, Showa University School of Medicine, Tokyo, Japan;* [4]*Institute of Molecular Oncology, Showa University School of Medicine, Tokyo, Japan;* [5]*Department of Dermatology, Showa University Fujigaoka Hospital, Yokohama, Japan;* [6]*Department of Respiratory Medicine, Showa University Fujigaoka Hospital, Yokohama, Japan and* [7]*Johns Hopkins University, Asthma and Allergy Center, Baltimore, Maryland, USA*

Correspondence: Dr Hideaki Watanabe, Department of Dermatology, Showa University School of Medicine, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8666, Japan. E-mail: aki2516w@cj8.so-net.ne.jp

Abbreviations: ERK, extracellular signal-regulated kinase; NHEK, normal human epidermal keratinocyte

Received 31 December 2007; revised 27 July 2008; accepted 30 July 2008; published online 2 October 2008

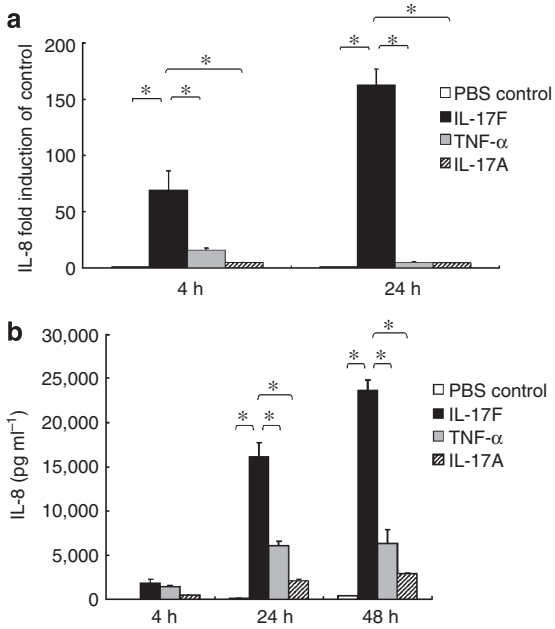

Figure 1. IL-17F induces IL-8 production by NHEKs. (**a**) *IL-8* gene expression by IL-17F in NHEKs. Total RNA was extracted from the cell lysates 4 and 24 hours after stimulation with 100 ng ml^{-1} IL-17F, 100 ng ml^{-1} TNF-α, 100 ng ml^{-1} IL-17A, and PBS control. The levels of mRNA for *IL-8* were calculated as the fold induction compared with the PBS control using real-time PCR. (**b**) Analysis of IL-8 production in NHEKs stimulated with IL-17F (100 ng ml^{-1}), TNF-α (100 ng ml^{-1}), IL-17A (100 ng ml^{-1}), and PBS control. IL-8 protein release in the supernatant was determined by ELISA as described in ''Materials and Methods''. Results represent mean ± SEM from at least three independent experiments. *$P<0.05$ was considered significant.

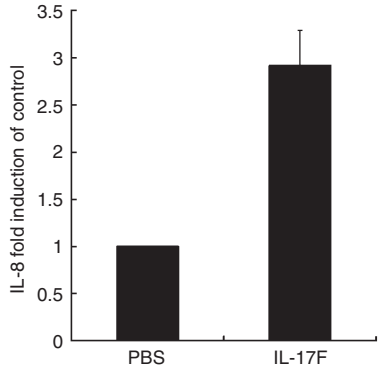

Figure 2. *IL-8* gene expression in the mouse skin induced by IL-17F. Ear tissue from the IL-17F-treated and PBS control mice were collected 24 hours following intradermal injection and total RNA was isolated from each ear specimen. The primer sequence was as follows: upstream 5′-ATGGCTGGG ATTCACCTCAA-3′, downstream 5′-AAGCCTCGCGACCATTCTT-3′. The expression of *IL-8* mRNA 24 hours was 2.9-fold higher than that in the control group. Results represent mean ± SEM from three independent experiments.

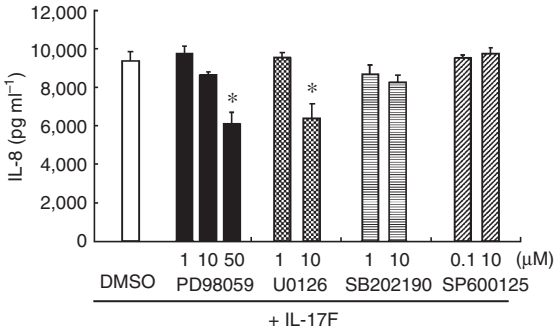

Figure 3. Effect of inhibitors on IL-8 protein production in NHEKs. The cells were preincubated with varying concentrations of PD98059, U0126, SB202190, SP600125, or Me_2SO vehicle for 1 hour, followed by stimulation with IL-17F (100 ng ml^{-1}) for 24 hours. Results represent mean ± SEM from three independent experiments. *$P<0.05$ was considered significant versus IL-17F-stimulated cells without addition of inhibitors.

IL-17F induces IL-8 production by NHEKs

We next investigated whether IL-17F-induced IL-8 protein production by keratinocytes. IL-17F elicited a time-dependent increase of IL-8 protein levels from 4 to 48 hours compared with TNF-α, IL-17A, and the phosphate-buffered saline (PBS) control (Figure 1b). The levels of secreted IL-8 were increased further at 48 hours. The amount of IL-8 stimulated by IL-17F at 48 hours was 3.7-fold higher and 8.3-fold higher than that stimulated by TNF-α and IL-17A, respectively. These findings demonstrated that the level of IL-8 protein correlated well with the IL-8 mRNA level. Thus, the results indicated that IL-17F is a strong inducer of IL-8 in keratinocytes.

IL-8 gene expression in the mouse skin induced by IL-17F

To further confirm the *IL-8* gene expression in the mouse skin after IL-17F injection, we examined whether IL-17F increased the expression of mRNA for *IL-8* using quantitative reverse transcription real-time PCR. The expression of *IL-8* mRNA 24 hours after injection was 2.9-fold higher than that in the control group (Figure 2).

ERK1/2 is involved in IL-17F-induced IL-8

A recent study has demonstrated the involvement of extracellular signal-regulated kinase (ERK) 1/2 kinase, but not P38 or c-Jun N-terminal kinase, in IL-17F- induced IL-8 production by primary bronchial epithelial cells and human umbilical vein endothelial cells (Kawaguchi *et al.*, 2002). Therefore, we examined whether activation of ERK1/2 was necessary for stimulation of IL-8 production in NHEKs. The NHEKs were preincubated with various concentrations of the mitogen-activated protein kinase kinase inhibitors, PD98059 and U1026, a p38 inhibitor, SB202190, and a c-Jun N-terminal kinase inhibitor, SP600125. As demonstrated in Figure 3, both PD98059 and U0126 partially, but significantly inhibited the production of IL-8 in a dose-dependent manner, whereas pretreatment of the cells with SB202190, SP600125, and the control (Me_2SO) did not affect IL-8 protein release in NHEKs.

Treatment with IL-17F induces ERK1/2 phosphorylation in the mouse skin

Ear tissue from the IL-17F-treated mice was collected 0.5, 1, and 2 hours after intradermal injection (100 ng in 50 μl of

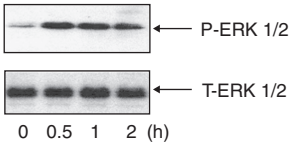

Figure 4. Treatment with IL-17F induces ERK1/2 phosphorylation in the mouse skin. Ear tissue samples collected at 0.5, 1, and 2 hours following IL-17F injection (100 ng in 50 μl of PBS) were prepared. Western blot analysis of phosphorylated and total ERK1/2 was performed on these skin samples. The activation of ERK1/2 was seen at 0.5, 1, and 2 hours after injection. The results shown are representative of three separate experiments.

PBS). Western blot analysis of phosphorylated and total ERK1/2 was performed on these skin samples. As shown in Figure 4, the activation of ERK1/2 was seen at 0.5, 1, and 2 hours after injection. These *in vitro* (Figure 3) and *in vivo* experiments (Figure 4) indicate that ERK1/2 signaling can contribute to the upregulation of IL-17F-induced IL-8 synthesis.

Induction of neutrophil accumulation in mouse skin by IL-17F injection

To examine the effect of local immune change by IL-17F, we intradermally injected recombinant mouse (rm) IL-17F, TNF-α, or IL-17A into mice. Histological examination of H-E-stained sections showed that the skin of mice injected with rm IL-17F showed marked infiltration of inflammatory cells in the dermis (Figure 5a) compared with that seen in the TNF-α injected mice (Figure 5c), IL-17A mice (Figure 5d), and control mice (Figure 5e). Higher magnification (Figure 5b) showed that the skin injected with IL-17F had neutrophil infiltration with fewer mononuclear cells in the dermis. No eosinophils were observed in all groups. Furthermore, we also examined whether anti-IL-8 Ab blocked the infiltration of inflammatory cells in IL-17F-treated group. As shown in Figure 5f and g, inflammatory cells in dermis showed significant but not complete blocking by anti-IL-8 antibody. These findings suggest that IL-8 released by keratinocytes in IL-17F-injected skin may be responsible for accumulation of neutrophils.

Detection of IL-17F in the skin of psoriasis patients

To examine the IL-17F expression in skin disease, we retrospectively examined skin biopsy samples from psoriasis patients. A total of 10 biopsy specimens from psoriasis vulgaris patients were studied to examine the expression of IL-17F using western blotting analysis. IL-17F protein was clearly detected in the psoriatic lesional skin compared with nonlesional skin from the same patients (Figure 6a). Furthermore, we quantified the IL-17F protein level by ELISA method. IL-17F level in the psoriatic lesional skin was higher than in nonlesional psoriatic skin. However, low level of IL-17F protein was also detected in nonlesional psoriatic skin (Figure 6b).

DISCUSSION

Several studies demonstrating IL-8 localization in psoriatic lesions at the mRNA or protein level have suggested that keratinocytes are a major source of IL-8 (Gillitzer *et al.*, 1991; Nickoloff *et al.*, 1991; Antilla *et al.*, 1992; Ozawa *et al.*, 2005). Moreover, Ghoreschi *et al.* (2003) showed that improvement of psoriasis correlates with suppression of IL-8 in the skin. IL-17F has been linked to tissue neutrophil recruitment through the induction of IL-8 (Kawaguchi *et al.*, 2004). In this study, IL-17F elicited a time-dependent increase in IL-8 protein, and the findings demonstrated that the level of IL-8 protein was correlated well with the levels of *IL-8* mRNA. Interestingly, the amount of IL-8 protein at 48 hours was 3.7-fold higher than that of TNF-α. It is well known that TNF-α is a strong inducer of IL-8 in NHEKs (Larsen *et al.*, 1989; Barker *et al.*, 1990). In fact, the cytokine network involved in psoriasis has TNF-α at center stage as a key primary cytokine involved in the induction and maintenance of plaques (Gottlieb *et al.*, 2005). Our study showed IL-17F is a stronger inducer of IL-8 in comparison to TNF-α. This provides evidence supporting the role of IL-17F in inflammatory skin diseases, such as psoriasis, and that IL-17F is an efficient inducer of IL-8 release by keratinocytes. Moreover, the production of IL-8 protein stimulated by IL-17F was also much higher than that stimulated by IL-17A. Similarly, in cystic fibrosis, in which pathogenesis is associated with neutrophils and IL-8, the levels of IL-17F were higher than IL-17A in sputum from the patients (McAllister *et al.*, 2005). This would indicate that IL-17F has diverse biological functions compared with IL-17A, even though both of them are produced by Th17 cells.

We also demonstrated the involvment of ERK1/2 kinase, but not p38 or c-Jun N-terminal kinase, in IL-17F-induced IL-8 production in NHEKs and mouse skin. This result is in agreement with recent observations obtained using primary bronchial epithelial cells, human umbilical vein endothelial cells, and human gastric epithelial cells (Kawaguchi *et al.*, 2002; Sebkova *et al.*, 2004). However, induction of IL-8 was not completely inhibited by the ERK1/2 kinase inhibitors PD98059 and U0126, suggesting that additional signaling pathways are involved in cytokine induction by IL-17F in keratinocyte. Hwang *et al.* (2004) reported that IL-17F-mediated induction of IL-8 may be transduced, in part, via activation of PI3-kinase/Akt pathway and NF-κB. Further research will focus on uncovering the signaling pathway of IL-17F.

It has been reported that there is an *in vivo* role of human IL-17F in recruiting neutrophils into the pulmonary mucosa in mice after adenoviral gene transfer (Hurst *et al.*, 2002), and IL-17F stimulated bronchial epithelial cells to produce IL-8 (Kawaguchi *et al.*, 2001; Oda *et al.*, 2005). In our study, the mouse skin intradermally injected with rm IL-17F showed marked neutrophil infiltration in the dermis with fewer mononuclear cells. Moreover, the neutrophil infiltration was significantly blocked by anti-IL-8 antibody. These findings suggest that neutrophilia in the dermis was induced via induction of IL-8 in keratinocytes stimulated by IL-17F. Thus, the IL-17F-IL-8 axis may be important in the pathophysiologic events in psoriasis.

In summary, we have investigated the effect of IL-17F in skin disease using *in vitro* and *in vivo* approaches. Recent

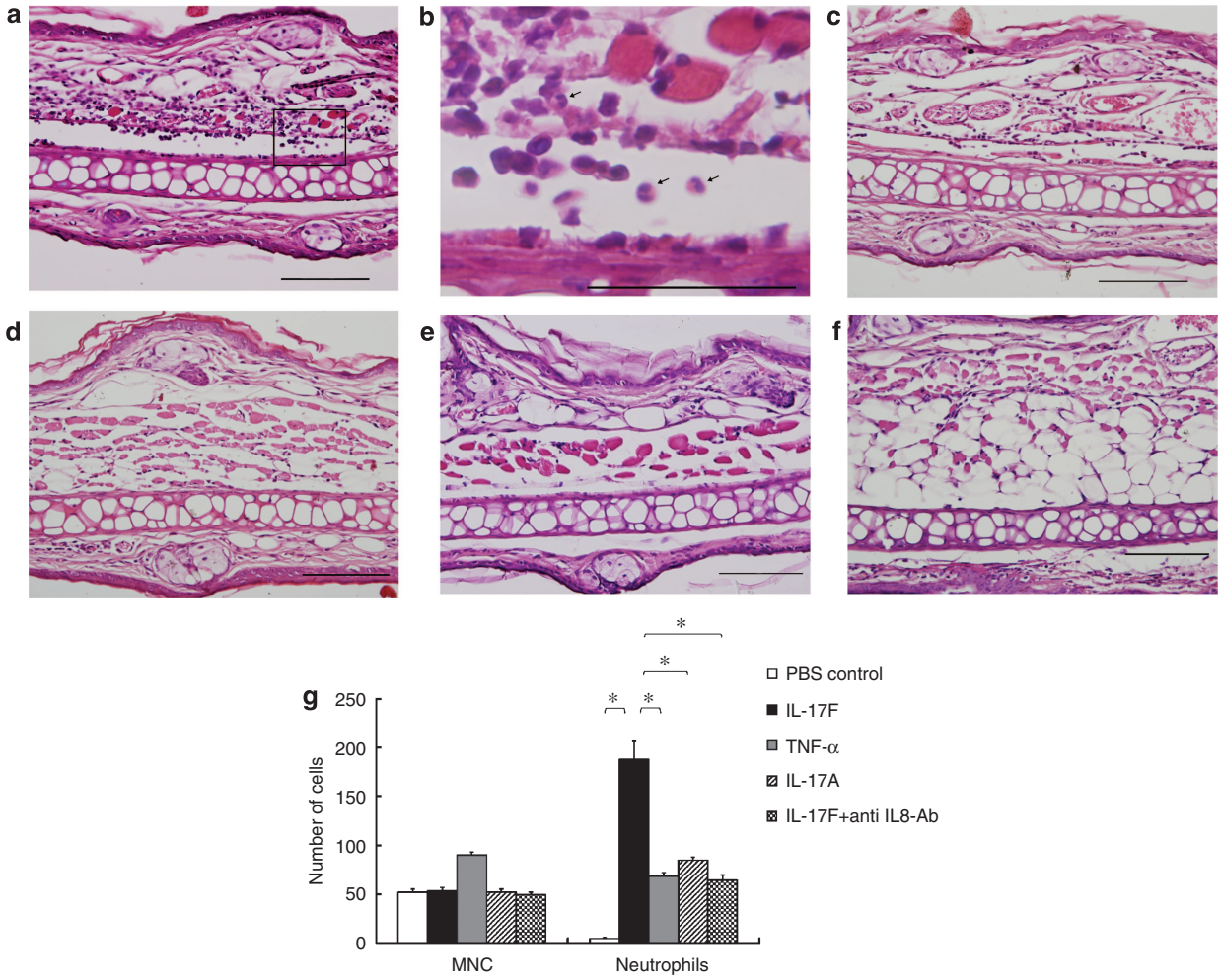

Figure 5. Histologic examination. Histopathological findings 48 hours after intradermal injection of (**a**) IL-17F, (**c**) TNF-α, (**d**) IL-17A, and (**e**) PBS control. Bar = 100 μm. (**b**) High magnification of **a**. Infiltrating cells in the dermis consisted mainly of neutrophils (arrow). Bar = 50 μm. (**g**) A cell count was performed as described in ''Materials and Methods'', and the density of dermal infiltration was expressed as mononuclear cells, neutrophils, eosinophils, and total number of cells. No infiltrating eosinophils were seen in both the IL-17F-treated and other groups. The number of neutrophils in the dermis was significantly increased by injection of recombinant mouse IL-17F compared with the control group (*$P<0.05$) and the inflammatory cells in the dermis were significantly but not completely blocked by anti-IL-8 antibody (*$P<0.05$) (**f**, **g**). Bar = 100 μm.

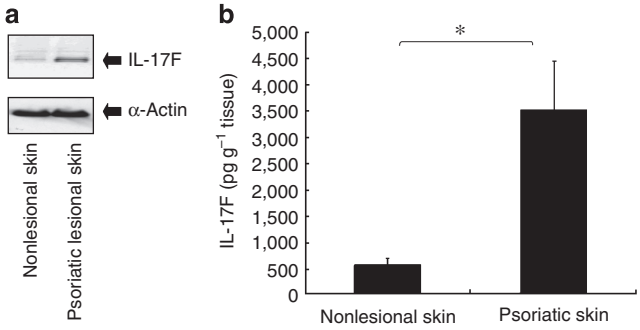

Figure 6. IL-17F protein in the skin of psoriasis patients. (**a**) Representative results from analysis of IL-17F protein production in the nonlesional and lesional skin of psoriasis patients. Skin specimens were homogenized and the supernatants were examined by western blotting. (**b**) Protein level of IL-17F in the psoriatic lesional skin was higher than in nonlesional psoriatic skin (*$P<0.05$). Low level of IL-17F protein was also detected in nonlesional psoriatic skin. The results were expressed as the mean ± SEM ($n=5$). *$P<0.05$ was considered significant.

studies suggest that Th17 cytokines are important in psoriasis (Blauvelt, 2007; Nickoloff, 2007; Zaba *et al.*, 2007; Haider *et al.*, 2008). Wilson *et al.* (2007) showed *IL-17F* mRNA had significantly higher expression in lesional skin than in nonlesional or healthy skin. Moreover, they explained that low level of *IL-17F* was detected in psoriatic nonlesional skin. Our study confirmed this finding by protein level. Further investigation of IL-17F may open new avenues for the development of biological therapies for psoriasis.

MATERIALS AND METHODS

All experiments were conducted in accordance with the Declaration of Helsinki Principles.

Cell culture and human recombinant IL-17F

NHEKs were purchased from Cambrex Bio Science Walkersville Inc. (Walkersville, MD) and used between passages 2 and 3. The cells were maintained in accordance with the supplier's protocols.

NHEKs were grown using a KGM-2 Bullet Kit (Cambrex Bio Science Walkersville Inc.). Human recombinant IL-17F was generated as reported previously (Kawaguchi *et al.*, 2001). Endotoxin levels were tested by using Kinetic-QCL Chromogenic Limulus amebocyte lysate (Bio Whittaker, Walkersville, MD), but were undetectable. The cells were treated with IL-17F, TNF-α (R&D Systems, Minneapolis, MN), IL-17A (R&D Systems), or the same volume of PBS for various time periods.

Animals

Female BALB/c mice were obtained from the Sankyo Laboratory (Saitama, Japan) and used at 8–10 weeks of age. Five mice were used in each experimental group, and each experiment was repeated at least three times. The animal protocol was approved by the institutional Animal Care and Use Committee of Showa University.

Treatment with IL-17F

To examine the effect of local administration of IL-17F to the mouse skin, we injected rm IL-17F (100 ng in 50 μl of PBS) (R&D Systems) intradermally into one side of each ear of the mouse using a 30-gauge needle. An equal volume of PBS was injected into the ears of the control group. In some experiments described below, rmTNF-α (100 ng in 50 μl of PBS), rm IL-17A (100 ng in 50 μl of PBS), rm IL-17F plus anti-human IL-8 antibody (100 ng rm IL-17F and 100 ng anti-human IL-8 Ab in total 50 μl of PBS) (all R&D Systems) were also intradermally administrated. The mice were anesthetized with pentobarbital sodium (50 mg kg^{-1} IP; Abbott Laboratories, IL).

Histologic examination

Ear tissues from the IL-17F, TNF-α, IL-17A, and control mice were collected 48 hours after intradermal injection. We also injected anti-IL-8 antibody to examine whether IL-8 antibody blocked the infiltration of inflammatory cells by IL-17F. Slides were prepared for routine histology and stained with hematoxylin and eosin. The slides were examined by light microscopy and the histological changes were compared between the groups. The density of the dermal infiltration was expressed as mononuclear cells, neutrophils, eosinophils, and total number of cells. A cell count was performed on each slide in ten random high-power fields with a ×40 objective (net magnification ×400) in order to calculate the mean dermal cellular infiltrate in areas of each ear, as reported previously (Kondo *et al.*, 1995; Watanabe *et al.*, 2004).

Skin biopsy

We retrospectively reviewed skin biopsies from 10 patients who had been diagnosed as having psoriasis vulgaris by both dermatologists and pathologists between 2000 and 2007. All the patients had been referred to Showa University Hospital. Oval-shaped skin biopsy samples had been obtained from the lesional area including the adjacent normal skin. Slides were prepared for routine histology and stained with hematoxylin and eosin. Moreover, small pieces of skin from the lesional and normal areas were stored at −70 °C for western blot analyses and ELISA.

All subjects were unrelated Japanese individuals and gave written informed consent for use of their samples. The study was approved by the Ethics Committee of Showa University School of Medicine.

Quantitative reverse-transcription and real-time PCR

Total RNA was extracted using an acid guanidinium thiocyanate–phenol–chloroform method from 1×10^6 NHEKs cells at 4 and 24 hours after stimulation with 100 ng ml^{-1} IL-17F, 100 ng ml^{-1} TNF-α, 100 ng ml^{-1} IL-17A, and the same volume of PBS as a control. cDNAs were synthesized from isolated RNA templates with a High-Capacity cDNA Archive Kit (Applied Biosystems, Tokyo, Japan). Pre-designed TaqMan probe sets for *IL-8* were purchased from Applied Biosystems. Each probe has a fluorescent reporter dye (FAM) linked to its 5′ end, and a downstream quencher dye (TAMRA) linked to its 3′ end. We used a TaqMan Ribosomal RNA probe, which is labeled with a fluorescent reporter dye (VIC), as an internal control. Each reaction was performed in a 25-μl volume containing 2× Universal Master Mix (Applied Biosystems), primers, labeled probes and 50 ng cDNA. Amplification conditions consisted of 40 cycles of 95 °C for 15 seconds and 60 °C for 1 minutes after incubation at 95 °C for 10 minutes. Amplification and fluorescence measurements were carried out during the elongation step with an ABI PRISM 7700 Sequence Detection System (Applied Biosystems).

Ear tissue from the IL-17F-treated and PBS control mice were collected 24 hours following intradermal injection and total RNA was isolated from each ear specimen with an acid guanidinium thiocyanate–phenol–chloroform method. The primer sequence for *IL-8* gene evaluation was decided as Heishi *et al.* (2003) and Inoue *et al.* (2007) performed in their experiment using mouse model (Figure 2). Data are shown as fold induction relative to control cells treated with PBS. All PCRs were performed in triplicate.

Levels of IL-8 protein

IL-8 protein levels in the collected supernatants of stimulated NHEKs were determined with a commercially available ELISA kit (R&D Systems) according to the manufacturer's instructions. Cell supernatants were harvested from cultures in the absence or presence of 100 ng ml^{-1} IL-17F, 100 ng ml^{-1} TNF-α, 100 ng ml^{-1} IL-17A, and the same volume of PBS, 4, 24, or 48 hours after stimulation. The amount of secreted IL-8 was determined by ELISA. Each supernatant was analyzed in duplicate.

Effect of inhibitors on the expression of IL-8

For analysis of the effect of kinase inhibitors, the cells were treated in the presence or absence of the following inhibitors at various doses: mitogen-activated protein kinase kinase 1/2 inhibitors, PD98059 (Calbiochem, La Jolla, CA) and U0126 (New England Bio Labs, Beverly, MA); p38 inhibitor, SB202190 (Calbiochem); a c-Jun N-terminal kinase inhibitor, SP600125 (Calbiochem); and a vehicle control, dimethyl sulfoxide (Me_2SO) for 1 hour before treatment with IL-17F (100 ng ml^{-1}). We decided the concentrations of various kinase inhibitors according to the previous studies (Davies *et al.*, 2000; Zhu *et al.*, 2006). The cell supernatants were harvested at 4 and 24 hours after stimulation for analysis by ELISA. IL-8 protein levels in the supernatants were determined as described above.

IL-17F-induced ERK1/2 activation in mouse skin

Ear tissue samples collected at 0.5, 1, and 2 hours following IL-17F injection (100 ng in 50 μl of PBS) for detection of ERK1/2 were prepared and homogenized in lysis buffer containing 50 mM Tris (tris(hydroxymethyl)-aminomethane) (pH 7.4), 150 mM NaCl, 0.02 M phenylmethlsulfonyl fluoride, 50 mg ml^{-1} leupeptin, and 50 mg ml^{-1}

aprotinin (all Sigma, St Louis, MO). Supernatants were collected after centrifugation (14,000 r.p.m., 20 minutes) and stored at −70 °C until analysis. Chemiluminescence luminol regent (Santa Cruz Biotechnology, CA) was used for detection.

Protein analysis for skin samples from psoriasis patients

Skin biopsy samples obtained from psoriasis patients were homogenized in lysis buffer. A total of 50 μg of protein per sample was analyzed by denaturing sodium SDS–PAGE and immunoblotted with a polyclonal antibody against human IL-17F (MBL, Nagoya, Japan). IL-17F protein levels in the supernatants were quantified by commercially available ELISA kit (R&D Systems). Each supernatant was analyzed in duplicate. Results were expressed as IL-17F concentration in pg g^{-1} tissue for homogenates.

Data analysis

The statistical significance of differences was determined by analysis of variance. Data are expressed as the mean ± SEM from independent experiments. Any difference with *P*-values of less than 0.05 was considered significant. When analysis of variance indicated a significant difference, the Scheffe F-test was used to determine the difference between groups.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This study was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan, and, in part, by National Institutes of Health (USA) grant RO1 AI-052468.

REFERENCES

Albanesi C, Cavani A, Girolomoni G (1999) IL-17 is produced by nickel-specific T lymphosytes and regulates ICAM-1 expression and chemokine production in human keratinocytes: synergistic or antagonist effect with IFN-γ and TNF-α. *J Immunol* 162:494–502

Albanesi C, Scarponi C, Cavani A, Federici M, Nasorri F, Girolomoni G (2000) Interleukin-17 is produced by both Th1 and Th2 lymphocytes, and modulates interferon-γ and interleukin-4-induced activation of human kertinocytes. *J Invest Dermatol* 115:81–7

Anttila HS, Reitamo S, Erkko P, Ceska M, Moser B, Baggiolini M (1992) Interleukin-8 immunoreactivity in the skin of healthy subject and patients with palmoplantar pustulosis and psoriasis. *J Invest Dermatol* 98:96–101

Barker JN, Sarma V, Mitra RS, Dixit VM, Nickoloff BJ (1990) Marked synergism between tumor necrosis factor-alpha and interferon-gamma in regulation of keratinocyte-derived adhesion molecules and chemotactic factors. *J Clin Invest* 85:605–8

Blauvelt A (2007) New concepts in the pathogenesis and treatment of psoriasis: key role for IL-23, IL-17A and TGF-b1. *Expert Rev Dermatol* 2:69–78

Davies SP, Reddy H, Caivano M, Cohen P (2000) Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J* 351:95–105

Duan H, Koga T, Kohda F, Hara H, Urabe K, Furue M (2001) Interleukin-8-positive neutrophils in psoriasis. *J Dermatol Sci* 26:119–24

Ghoreschi K, Thomas P, Breit S, Dugas M, Mailhammer R, van Eden W *et al.* (2003) Inreleukin-4 therapy of psoriasis induces Th2 responses and improves human autoimmune disease. *Nat Med* 9:40–6

Gillitzer R, Berger R, Mielke V, Muller C, Wolff K, Stingl G (1991) Upper keratinocyte of psoriatic skin lesions express high level of NAP-1/IL-8 mRNA *in situ*. *J Invest Dermatol* 97:73–9

Gottlieb AB, Chamian F, Masud S, Cardinale I, Abello MV, Lowes MA *et al.* (2005) TNF inhibition rapidly down-regulates multiple proinflammatory pathways in psoriasis plaques. *J Immunol* 175:2721–9

Haider AS, Lowes MA, Suarez-Farinas M, Zaba LC, Cardinale I, Khatcherian A *et al.* (2008) Identification of cellular pathways of ''Type 1'', Th17 T cells, and TNF- and inducible nitric oxide synthase-producing dendritic cells in autoimmune inflammation trough pharmacogenomic study of cyclosporine A in psoriasis. *J Immunol* 180:1913–20

Heishi M, Imai Y, Katayama H, Hashida R, Ito M, Shinagawa A *et al.* (2003) Gene expression analysis of atopic dermatitis-like skin lesions induced in NC/Nga mice by mite antigen stimulation under specific pathogen-free conditions. *Int Arch Allergy Immunol* 132:355–63

Hurst SD, Muchamuel T, Gorman DM, Gilbert JM, Clifford T, Kwan S *et al.* (2002) New IL-17 family members promote Th1 or Th2 responses in the lung: *in vivo* function of the novel cytokine IL-25. *J Immunol* 169:443–53

Hwang SY, Kim JY, Kim KY, Park MK, Moon Y, Kim WU *et al.* (2004) IL-17 induces production of IL-6 and IL-8 in rheumatoid arthritis synovial fibroblasts via NF-kB- and PI3kinase/Akt-dependent pathways. *Arthritis Res Ther* 6:R120–8

Hymowitz SG, Filvaroff EH, Yin JP, Lee J, Cai L, Risser P *et al.* (2001) IL-17s adopt a cystine knot fold: structure and activity of a novel cytokine, IL-17F, and implications for receptor binding. *EMBO J* 20:5332–41

Inoue R, Otsuka M, Nishio A, Ushida K (2007) Primary administration of *Lactobacillus johnsonii* NCC533 in weaning period suppresses the elevation of proinflammatory cytokine and CD86 gene expression in skin lesions in NC/Nga mice. *FEMS Immunol Med Microbiol* 50:67–76

Kawaguchi M, Kokubu F, Odaka M, Watanabe S, Suzuki S, Ikei K *et al.* (2004) Induction of granulocyte-macrophage colony-stimulating factor by a new cytokine, ML-1 (IL-17F), via Raf I-MEK-ERK pathway. *J Allergy Clin Immunol* 114:444–50

Kawaguchi M, Ounchic LF, Huang SK (2002) Activation of extracellular signal-regulated kinase (ERK) 1/2, but not p38 and c-Jun N-terminal kinase, is involved in signaling of a novel cytokine, ML-1. *J Biol Chem* 277:15229–32

Kawaguchi M, Onuchic LF, Li X-D, Essayan D, Schroeder J, Xiao H-Q *et al.* (2001) Identification of a novel cytokine, ML-1, and its expression in subjects with asthma. *J Immunol* 167:4430–5

Kondo S, Pastore S, Fujisawa H, Sauder DN (1995) Interleukin-1 receptor antagonist suppresses contact hypersensitivity. *J Invest Dermatol* 105:334–8

Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K (1989) Production of interleukin-8 by human dermal fibroblast and keratinocytes in response to interleukin-1 or tumor necrosis factor. *Immunology* 68:31–6

McAllister F, Henry A, Kreindler JL, Dubin PJ, Ulrich L, Steele C *et al.* (2005) Role of IL-17A, IL-17F, and the IL-17 receptor in regulating growth-related oncogene-alpha and granulocyte colony-stimulating factor in bronchial epithelium: implications for airway inflammation in cystic fibrosis. *J Immunol* 175:404–12

Nickoloff BJ (2007) Cracking the cytokine code in psoriasis. *Nat Med* 13:242–4

Nickoloff BJ, Karabin GD, Barker JN, Griffiths CE, Sarma V, Mitra RS *et al.* (1991) Cellular localization of interleukin 8 and its inducer, tumor necrosis factor alpha in psoriasis. *Am J Pathol* 138:129–40

Nickoloff BJ, Qin JZ, Nestle FO (2007) Immunopathogenesis of psoriasis. *Clin Rev Allergy Immunol* 33:5–56

Oda N, Canelos PB, Essayan DM, Plunkett BA, Myers AC, Huang SK (2005) Interleukin-17F induces pulmonary neutrophilia and amplifies antigen-induced allergic response. *Am J Respir Crit Care Med* 171:12–8

Ozawa M, Terui T, Tagami H (2005) Localization of IL-8 and complement components in lesional skin of psoriasis vulgaris and pustulosis palmaris et plantaris. *Dermatology* 211:249–55

Sebkova L, Pellicano A, Monteleone G, Grazioli B, Guarnieri G, Imeneo M *et al.* (2004) Extracellular signal-regulated protein kinase mediates interleulin 17 (IL-17)-induced IL-8 secretion in Helicobacter pylori-infected human gastric epithelial cells. *Infect Immun* 72:5019–26

Starnes T, Robertson MJ, Sledge G, Kelich S, Nakshatri H, Broxmeyer HE *et al.* (2001) IL-17F, a novel cytokine selectivity expressed in activated T cells and monocytes, regulates angiogenesis and endothelial cell cytokine production. *J Immunol* 167:4137–40

Steinman L (2007) A brief history of Th17, the first major revision in the Th1/Th2 hypothesis of T cell-mediated tissue damage. *Nat Med* 13:139–45

Sticherling M, Bornscheuer E, Schroder JM, Christophers E (1991) Localization of neutrophil-activating peptide-1/interleukin-8 immunoreactivity in normal and psoriatic skin. *J Invest Dermatol* 96:26–30

Takematsu H, Tagami H (1993) Quantification of chemotactic peptides (C5a anaphylatoxin and IL-8) in psoriatic lesional skin. *Arch Dermatol* 129:74–80

Teunissen MB, Koomen CW, de Waal Malefyt R, Wierenga EA, Bos JD (1998) Interkeukin-17 and interferon-gamma synergize in the enhancement of proinflammatory cytokine production by human keratinocytes. *J Invest Dermatol* 111:645–9

Toda M, Leung DY, Molet S, Boguniewicz M, Taha R, Christodoulopoulos P *et al.* (2003) Polarized *in vivo* expression of IL-11 and IL-17 between acute and chronic skin lesions. *J Allergy Clin Immunol* 111:875–81

van Beelen AJ, Teunissen MB, Kapsenberg ML, de Jong EC (2007) Interleukin-17 in inflammatory skin disorders. *Curr Opin Allergy Clin Immunol* 7:374–81

Watanabe H, Mamelak AJ, Wang B, Howell BG, Freed I, Esche C *et al.* (2004) Anti-vascular endothelial growth factor receptor-2 (Flk-2/KDR) antibody suppresses contact hypersensitivity. *Exp Dermatol* 13:671–81

Wilson NJ, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD *et al.* (2007) Development, cytokine profile and function of human inteleukin 17-producing helper T cells. *Nat Immunol* 8:950–7

Zaba LC, Cardinale I, Gilleaudeau P, Sullivan-Whalen M, Suarez-Farinas M, Fuentes-Duculan J *et al.* (2007) Amelioration of epidermal hyperplasia by TNF inhibition is associated with reduced Th17 responses. *J Exp Med* 204:3183–94

Zheng Y, Danilenko DM, Valdez P, Kasman I, Eastham-Anderson J, Wu J *et al.* (2007) Interleukin-22, a Th17 cytokine, mediates IL-23-induced dermal inflammation and acanthosis. *Nature* 445:648–51

Zhu M, Zhang Y, Bowden GT (2006) Involvement of mitogen-activated protein kinases and protein kinase C in regulation of antioxidant response element activity in human keratinocytes. *Cancer Lett* 244:220–8